INHIBITORY G PROTEINS IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

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The rectal gland of the shark increases its rate of chloride secretion in response to vasoactive intestinal peptide (VIP) and this stimulation is inhibited by somatostatin (Stoff JS, et al. Am. J. Physiol. 1979, 237:F138-44). Since the effect of VIP is mediated by adenylate cyclase (Stoff JS, et al., ibid.) and somatostatin inhibits the VIP-induced accumulation of cAMP in cultured pituitary cells via an inhibitory regulatory G protein (Koch BD, et al. J. Biol. Chem. 1985, 260:13138-45) we studied the inhibitory G proteins in the rectal gland. G proteins are heterotrimeric proteins which are located in the membranes of cells, and serve to couple extracellular signals to intracellular responses. When the extracellular stimulus activates its receptor, the G protein binds GTP, and dissociates to form a free alpha subunit and a beta-gamma dimer. Although there are several types of G proteins, the best understood are the two types involved in the regulation of adenylate cyclase (AC); one of which activates the enzyme (designated Gs) and one which inhibits the enzyme (designated Gi).

We used monoclonal antibodies to identify by Western blot analysis the Gi proteins present in microsomal preparations of rectal gland and heart of the shark. We used two different antibodies, one that recognizes the common carboxy-terminal amino acid sequence [345-354] of Giα1 and Giα2 and another that recognizes the carboxy-terminal amino acid sequence [345-354] of Giα3. Both antibodies bound to ~41 kDa proteins in shark rectal gland and heart, as well as in outer medulla of the rat kidney, used as a control. These experiments confirm the presence of Gi proteins in the shark rectal gland and heart.

Gi proteins can be labeled by pertussis toxin, which catalyzes the ADP ribosylation of Gi only when the alpha unit is bound to the beta-gamma subunit and thereby renders it inactive. To determine whether Gi proteins mediate the inhibitory effect of somatostatin on the rectal gland we tested the effect of pertussis toxin on the ability of somatostatin to inhibit VIP stimulated short circuit current (Isc) in confluent monolayers of cultured rectal gland cells. (Isc is proportional to, and therefore measures indirectly, the net transport of chloride across the cultured epithelia.) We found that incubation of cells for 24 hours with pertussis toxin did not inhibit somatostatin's inhibition of the VIP-induced stimulation of Isc. This suggests that either, 1) somatostatin does not function via a pertussis toxin-sensitive Gi protein, which is counter to published reports on its mode of action, or 2) the pertussis toxin does not enter the rectal gland cell. We therefore utilized a plasma membrane preparation to study the role of Gi proteins in the rectal gland cell.

Membranes from shark rectal glands were prepared by homogenization and differential centrifugation, aliquoted and frozen at -70°C. Thawed membranes were incubated for 10 minutes at 20°C in shark Ringers (4 mg protein/ml) with or without hormone. For the ribosylation reaction, the pertussis toxin was first activated by incubation at 30°C for 30 minutes in 10 mM Hepes, pH=7.8; 0.2 mg/ml BSA; 5 mM dithiothreitol; 0.025% SDS; and 20 µg/ml of toxin. A 5 µl aliquot of the activated toxin was added to 10 µl of ribosylation mix and a 10 µL aliquot of the membrane incubation added to initiate the reaction. The final concentrations in the ribosylation reaction were: 20 µg/ml toxin; 1 mM EDTA; 10 mM thymidine; 5 mM NAD; 1 mM ATP; 0.1 mM GTP; 2.5 mM $MgCl_2$; ^{32}PNAD (20,000 cpm/pmol :); 1.6 mg/ml membrane protein. The mixtures were incubated for 20 minutes at 30°C, at which time the reactions were terminated by adding an equal volume of sample buffer and boiled for 3 minutes. A 20 µl sample was then subjected to SDS-PAGE on 12.5% gels (3% stacking gels). The gels were stained, dried, and used for autoradiography.

Figure 1 shows an autoradiograph of one of these gels.

In the absence of pertussis toxin there was nonspecific labeling of many membrane proteins. The inclusion of pertussis toxin in the ribosylation reaction resulted in the labeling of a protein having a molecular weight in the range of 40-45 kDa (the Gi subunit has a molecular weight of 40.5 kDa), and we are therefore confident that the band in the autoradiograph represents Giα. Incubation of the membranes with somatostatin (10^{-5} M) prior to ribosylation did not reduce the degree of labeling as would be expected if somatostatin causes dissociation of the Gi trimer. In additional experiments, we incubated the membranes with aluminum fluoride (10 mM NaF, 10 μM $AlCl_3$) which has been shown to cause dissociation of Gi, as well as 2-chloroadenosine (10^{-5} M) or neuropeptide Y (10^{-6} M). In no case did we observe significant decrease in the incorporation of ^{32}P-NAD into the toxin-specific band on the autoradiogram.

We conclude that although Gi proteins are present in the membranes of shark rectal gland cells, the extent of dissociation (if any) caused by inhibitors of adenylate cyclase is undetectable with the experimental protocol used in these experiments.

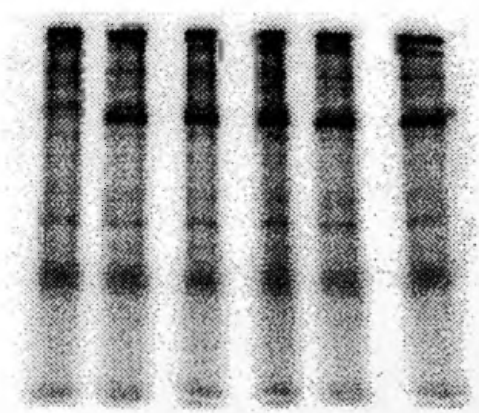

Figure 1. Autoradiograph of pertussis toxin labeling of Gi proteins in membranes of shark rectal gland. The first two lanes on the left are control membranes incubated without and with pertussis toxin. The following four lanes are incubated prior to ribosylation with somatostatin 10^{-5} M, AlF_4, 2-chloroadenosine 10^{-5} M and neuropeptide Y 10^{-6} M, respectively. Neither somatostatin nor any of the other treatments reduced the ribosylation of pertussis toxin-specific band.

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